

EXHIBIT F

AN 95140019 MEDICINE
 DN 95140019
 TI Dietary polyunsaturated fatty acids interfere with the insulin/glucose activation of L-type pyruvate kinase gene transcription.
 AU Liimatta M; Towle H C; Clarke S; Jump D B
 CS Department of Physiology, Michigan State University, East Lansing 48824.
 NC DK-43220 (NIDDK)
 DK-26919 (NIDDK)
 SO MOLECULAR ENDOCRINOLOGY, (1994 Sep) 8 (9) 1147-53.
 Journal code: NGZ. ISSN: 0888-8809.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199505
 AB L-type pyruvate kinase (L-PK) is a key glycolytic enzyme regulating the flux of metabolites through the pyruvate-phosphoenolpyruvate cycle (1). The regulation of L-PK is complex involving both hormones and nutrients. We have found that feeding rats diets containing polyunsaturated fatty acids (PUFA) significantly inhibits hepatic pyruvate kinase enzyme activity (> 60%) and suppresses mRNAPK abundance (> 70%). Studies with primary hepatocytes indicate that PUFA act directly on hepatocytes. Specifically, arachidonic (20:4, omega 6) and eicosapentaenoic (20:5, omega 3) acid suppressed both mRNAPK levels and the activity of a transfected PKCAT (-4300/+12) fusion gene by > 70%. This is due to an inhibition of the insulin/glucose-mediated transactivation of L-PKCAT. Deletion analysis localized PUFA-regulated cis-acting elements to a region within the L-PK proximal promoter, i.e. between -197 and -96 base pairs. This region binds two transcription factors involved in the hormone/nutrient regulation of L-PK gene transcription, i.e. a major late transcription factor-like factor and HNF-4. Linker scanning mutation analysis localized the PUFA-regulated cis-acting elements to the vicinity of the HNF-4 binding site. Thus, PUFA-regulated factors abrogate the insulin/glucose activation of L-PK gene transcription by targeting the HNF-4 elements. These studies suggest that PUFA may have significant effects on hepatic carbohydrate metabolism by inhibiting the L-PK side of the pyruvate-phosphoenolpyruvate cycle.
 CT Check Tags: Animal; Male; Support, U.S. Gov't, P.H.S.
 Arachidonic Acid: PD, pharmacology
 Base Sequence
 Cells, Cultured
 Depression, Chemical
 *Dietary Fats: PD, pharmacology
 Enzyme Induction: DE, drug effects
 *Fatty Acids, Unsaturated: PD, pharmacology
 *Fish Oils: PD, pharmacology
 *Glucose: PD, pharmacology
 Glycolysis: DE, drug effects
 *Insulin: PD, pharmacology
 Liver: DE, drug effects
 Liver: EN, enzymology
 Molecular Sequence Data
 Mutagenesis, Site-Directed
 Oleic Acids: PD, pharmacology
 *Pyruvate Kinase: BI, biosynthesis
 Pyruvate Kinase: GE, genetics
 Rats
 Rats, Sprague-Dawley
 Recombinant Fusion Proteins: BI, biosynthesis
 RNA, Messenger: BI, biosynthesis
 Transcription Factors: ME, metabolism
 *Transcription, Genetic: DE, drug effects
 5,8,11,14,17-Eicosapentaenoic Acid: PD, pharmacology
 RN 11061-68-0 (Insulin); 112-80-1 (Oleic Acid); 135845-90-8 (transcription factor HNF-4); 1553-41-9 (5,8,11,14,17-Eicosapentaenoic Acid); 50-99-7 (Glucose); 506-32-1 (Arachidonic Acid); 8002-50-4 (Menhaden oil)
 CN EC 2.7.1.40 (Pyruvate Kinase); 0 (Dietary Fats); 0 (Unsaturated); 0 (Fish Oils); 0 (Oleic Acids); 0 (R

Applicants: Jacob Bar-Tana
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 Exhibit F

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